Unravelling Structural Changes of Pea Globulins during Heat Treatment as Studied Using Atomistic Model-based SAXS

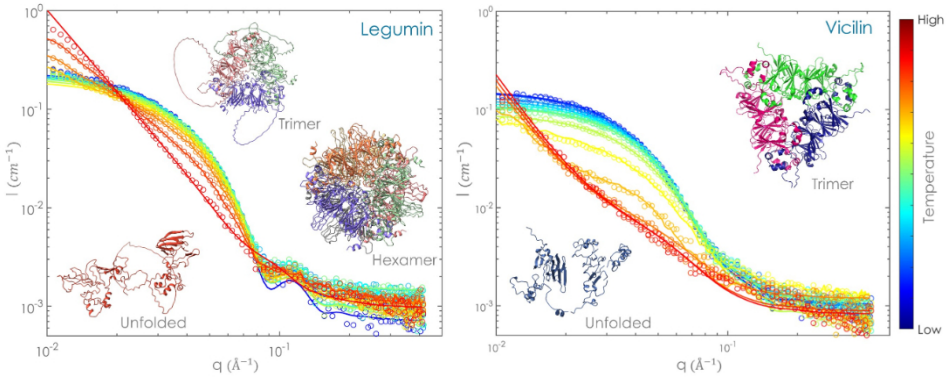
Antara Pal [1 2], Gökhan Ugur Atıl [3], Marcos Lopez Hernandez [4], Milena Corredig [3], Jan Skov Pedersen [4]

[1]Department of Biomedical Science, Malmö University, Malmö, Sweden
[2]Biofilms Research Center for Biointerfaces, Malmö University, Malmö, Sweden
[3]Department of Food Science, Aarhus University, Aarhus N, Denmark
[4]Department of Chemistry and Interdisciplinary Nanoscience Center, Aarhus University, Aarhus, Denmark

antara.pal@mau.se

Legumin and Vicilin are the most abundant storage proteins in pea (*Pisum sativum*), comprising approximately 53 ± 7% (w/w) of the total protein, and understanding both their native structures and thermally induced structural changes is essential for optimizing the design of plant-based products. Small-angle X-ray scattering (SAXS) is widely used to probe macromolecular structures in solution; however, conventional analyses often rely on low-resolution models that overlook important chemical and molecular details. To address this limitation, we present a methodology that integrates high-resolution SAXS modeling with AlphaFold-predicted oligomeric assemblies, enabling hypothesis-driven structural interpretation under complex and physiologically relevant conditions. Using pea globulins as a model system, isolates of Legumin and Vicilin were obtained through isoelectric precipitation followed by size exclusion chromatography fractionation, and their structures were examined in solution across a temperature range of 25–85 °C. At near-ambient temperatures, Vicilin adopted a trimeric structure, while Legumin existed as a mixture of trimers and hexamers. Association behavior was described using structure factors, and thermally unfolded states were modeled through rigid-body refinement. Upon heating, both proteins transitioned from native globular assemblies to unfolded conformations and, notably, did not revert to their native structures upon cooling. This hybrid approach, which bridges computational prediction with experimental scattering data, enables precise characterization of proteins across diverse conformational states and provides deeper insight into oligomerization, unfolding, and processing-related phase transitions. Overall, these findings advance our understanding of the thermal stability and structural plasticity of pea proteins, broaden the applicability of SAXS in studying protein dynamics in food systems, and offer valuable guidance for designing sustainable plant-based products as well as improving knowledge of other storage proteins from leguminous plants

Keywords:
Pea protein, SAXS, Structural changes



Temperature induced unfolding of Vicilin and Legumin studied using SAXS.